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EFFECT UPON PLATELETS AND ON BLOOD COAGULATION OF INJECTING FOREIGN PARTICLES INTO THE BLOOD STREAM.

By JOHN TAIT and A. R. ELVIDGE[1].

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It was pointed out by Tait(14) that many blood cells on contact with an area of non-greasy foreign material are, by capillary action, pulled progressively outwards over the foreign surface, like a drop of water on a stone. To such cells, which include mammalian platelets, many of the spindle-cells of amphibians and fishes, certain special cells of the blood of *Astacus* and of some other crustacea, and various corpuscles of mollusca, arthropoda and annulata, he applied the name *thigmocytes*. It is characteristic of thigmocytes that they are not distorted by contact with an oily or greasy surface; on the other hand, they are highly phagocytic towards minute particles of non-greasy matter.

In bloods that possess the property of true jelly coagulation, *e.g.* vertebrate blood or the blood of certain special crustacea (Heim(7); Tait(13)), the thigmocytes act as a source of thrombin. When, by capillary action, they have once been stretched beyond a certain limit, a sudden cytolytic disintegration occurs. Tait and Gunn(17) were able to show that cytolysis of the hyaline thigmocytes of *Astacus*, which occurs some 15–20 minutes after the blood is shed, is immediately followed by a coagulation of the surrounding plasma. Tait and Burke(15) demonstrated that mammalian platelets, having once attained a certain degree of expansion on a glass slide, suffer an equally abrupt disintegration and, like the hyaline thigmocytes of *Astacus*, discharge globules of thrombin into the plasma. That the stimulus which leads to cytolysis of thigmocytes is purely physical was proved by Tait and Green(16).

The fact that thigmocytes, without detriment to their stability, may engulf small particles of a given water-wettable material, while on a larger slab of the same material they suffer disaster in their efforts at phagocytosis, indicates that simple contact with foreign matter is not

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sufficient to determine disintegration of the cells. The structural collapse of these elements would appear to result from over-stretching. In the blood stream of some animals there are, however, cells of even more labile constitution, *e.g.* the explosive corpuscles of *Astacus* (Hardy(6); Tait and Gunn(17)) and of *Ligia* (Tait(12)), and possibly some of the cells of amphibians (Meves(9)). These appear to be still more sensitive to contact with water-wettable foreign matter. So delicate is their structural organisation that any little surface disturbance of a capillary kind leads forthwith to disintegration. In the nature of things such cells are not phagocytic (Tait and Gunn(17)). At the same time they, too, yield thrombin to the blood (cf. Tait(12), Tait and Gunn(17) and Tait and Green(16)).

In view of the thigmocytic reaction of mammalian platelets on a slab of water-wettable material and their corresponding power to ingest very minute particles without apparently suffering cytolysis, we arranged to inject into the blood stream minute particles of insoluble material which had been carefully graded according to size. It was possible, for example, that injection of particles of a size so small that they could be comfortably accommodated within the platelets might lead to no conspicuous reaction, whereas injection of particles of a diameter as large as red blood corpuscles might induce intravascular coagulation. It was eventually possible from the figure in Tait and Burke's(15) paper to estimate approximately the maximal area of expansion of both large and small platelets, which is some 100 sq. μ for the large and some 40 sq. μ for the small. These data were not, however, available when we began our experiments.

The only experiments known to us in which insoluble particles were deliberately added to blood with the object of inducing coagulation, are those of Rettger(10), who showed that "dust particles, loose sweepings, or linty shreds" added to centrifuged terrapin plasma induce a prompt coagulation. From the fact that both dust and long irrigated tissue extract produce similar coagulation in this reptilian plasma, Rettger was inclined to conclude, in opposition to the unvarying testimony of other workers, that tissue extract does not possess any peculiar or specific coagulating virtue. No authors appear to have investigated the possible coagulant effect of introducing foreign particles into the circulating current. At the same time the investigation is a necessary one, for such odd fragments of knowledge as we already possess in respect to platelets indicate high functional rank for these elements in a variety of processes not always suspected of having im-

mediate interrelation. In a succeeding paper (by J. T.) it will be shown anew how vital is the part they play in arresting hæmorrhage from a wound. They, and they alone, would appear to be involved in hæmophilia. In most thromboses they are the important agents. They play an important part in certain immunity reactions (Govaerts(5)). They are undoubtedly phagocytic, and, as phagocytes or as adhesive "grips" probably play a part in anchorage and disposal of blood-carried micro-organisms. Their special relation to the spleen has been shown by the work of Bedson(1). The all but universal occurrence of such elements, at least in animals possessed of a circulatory system[1], is a further indication of their general importance.

The platelets or hyaline thrombocytes of mammals are peculiar in at least two respects, in point of size and in apparent absence of a nucleus. Their minute size may be an adaptation to the diminutive proportions of invading organisms. Their non-nucleate or sub-nucleate condition, implying absence or defect of constructive metabolism, might suggest in their case a special adaptation as simple adhesive "grips" when anchorage of foreign circulating material is concerned.

Technical Details.

Preparation of quartz suspensions. The material chosen for preparation of the particles was fused quartz. (Were we to repeat the experiments we should use crystalline quartz, because of the possibility of subsequently detecting the particles in the tissues with a Nicol prism.) Transparent fragments, obtained from the Thermal Syndicate, Ltd., Wallsend-on-Tyne, England, were first subjected to a process of chemical purification for the purpose of removing grease and adherent soluble material. Between hard steel faces the dried pieces were broken into smaller fragments, which were then pounded to dust in a diamond mortar. The dust was now ground for 48 hours in a mechanically driven agate mortar. During the first 12 hours it remains heavy and sandy; later it commences to fluff up and becomes progressively lighter and more mobile. The ultimate fine product which, like "light magnesia," slides from side to side in the containing vessel, was stored in flasks of hard glass which had been chemically cleaned. Throughout these manipulations the quartz is never touched with the fingers, all precautions being taken to furnish clean surfaces of fracture or of attrition.

By levigation in high-grade conductivity water the particles were now assorted into graded sizes. The water of suspension had been sub-

[1] Hyaline thrombocytes do not seem to exist in the body-fluid of Echinodermata.

jected to five distillations, twice through glass, twice through special stills of "vitreosil" ware in which no cork or rubber joins were used. Before the fifth (and final) distillation a small quantity of potassium permanganate and sodium hydroxide was added to the distilling flask, in accordance with the recommendation of Kraus and Dexter(8). The object of this repeated distillation was to obtain water as free from electrolytes as possible.

The distilling flasks, provided with special necks, which rendered corks unnecessary, were of pyrex glass. The vessels for holding the ultimate conductivity water or quartz suspensions as the case might be, were of vitreosil or of clear fused quartz. All our grades of suspension showed Brownian movement, feeble, in the case of the coarse, and active in the case of the fine suspensions. The complete absence of agglutination and the continued presence of this Brownian movement over a period of days on end, sufficiently attested the absence of any contamination with electrolytes. After settling of a suspension complete dispersion at once occurred on shaking.

According to Stokes' law the speed of sedimentation, V, is directly proportional to F, the force of gravity, and inversely proportional to η, the viscosity of the dispersion medium, as well as to r, the radius of the particle, in accordance with the formula

$$V = F/6\pi\eta r.$$

Good agreement was found to obtain between the formula and the actual rate of sedimentation of the particles. At 18° C. particles just a little smaller than the red blood cells of the rabbit (6·5–7 μ in diameter) were, after 1½ hours, still held in the top inch of the fluid. Particles measuring from 2·5 to 3 μ in diameter remained in the top inch after 3 hours' settling. Particles less than 1·5 μ remained in the top inch after 24 hours' settling. Naturally it was necessary to levigate repeatedly in order to obtain uniform grades of suspension. Direct microscopic examination served as a complete check on uniformity. In what follows we shall designate particles 6–7 μ in diameter, with a calculated surface of some 113–154 sq. μ, as "coarse quartz"; particles from 3 to 3·5 μ, with a surface of some 13–39 sq. μ, as "medium quartz"; and particles less than 1·5 μ, with a calculated surface of 7 sq. μ and under, as "fine quartz."

The point of saturation of colloidal suspensions seems to be variable, the highest determination hitherto recorded being that of Zsigmondy(18), who obtained a permanent suspension containing 0·12 p.c. of gold sol.

On the other hand, Bredig(2) gave 0·014 p.c. as the maximum figure for a gold sol. On further concentration gold sols change colour, the change of colour indicating that agglutination has occurred. In our experiments, curiously enough, we could obtain no indication that mere high concentration tends towards agglutination. It was easily possible to hold 6 p.c. of the coarse suspension in complete dispersion for a time at least in the conductivity water. A concentration of 2·03 p.c. medium quartz without any agglutination was obtained. The fact that any single highly concentrated sample, after settling, could forthwith be redispersed by shaking, also proves that no effective agglutination ever occurred. The ease with which high concentration can be obtained speaks for a low surface energy. One might be led to suppose that the suspended quartz particles are surrounded by a hydrolytic layer.

Besides particles of quartz we used particles of Indian ink (already in finest suspension when purchased as "Pelican" brand), of barium sulphate and of carmine. After prolonged grinding in the agate mortar the last two substances fluff up in the same way as quartz. These other materials were used chiefly to check the results, for very finely divided quartz is not wholly insoluble. If an effect due to an injection of a quartz suspension can be reproduced when any kind of finely divided particles is substituted for the quartz, the conclusion is that simple particulate matter and not the presence of silicate in solution is responsible for the reaction.

Means of securing isotonicity. In some early experiments in which the salt constituents of buffered Ringer's fluid were added to the pure quartz suspension just before use, it was found that agglutination occurs so quickly as to block the needle of the hypodermic syringe, while the injected animals die at once of a coagulation which extends along the vein selected for injection to the right heart and thence along the pulmonary artery. A control experiment in which the individual salt constituents of buffered Ringer's fluid were separately added in their appropriate amounts to supplies of the quartz suspension, showed that the bivalent Ca-ion is by far the most effective agglutinant. An efficacious but somewhat tedious method of avoiding agglutination on addition of the salt is to take advantage of the protective action of serum proteins, thus. Equal quantities of fresh serum and of suspension are first mixed together, whereupon the appropriate quantities of NaCl, KCl, $CaCl_2$ and $NaHCO_3$ for the amount of quartz suspensions are stirred in. No agglutination occurs, the mixture being more than ever stable and slow of sedimentation. As serum, however, contains thrombin, which is

objectionable, the method we ultimately adopted and consistently used was to add 6 p.c. of pure glucose to the suspension just before injection into mammals and 4 p.c. before injection into frogs. By this means the fluid was rendered isotonic without the necessity of adding electrolytes; the presence of the glucose does not appear in any way to modify the result so far as the platelets are concerned.

Animals used and sites of injection. In some early experiments carried out on guinea-pigs the quartz was injected into the femoral vein. Owing to the difficulty of carrying out repeated injections, which necessitate anæsthesia and aseptic technique, guinea-pigs were discarded in favour of rabbits, into which the material may be easily introduced by way of the ear vein. A number of experiments were carried out on anæsthetised cats. It soon appeared that the needle-point may be introduced directly into the external saphenous vein of the cat without previous incision of the skin. By this device time is saved, while the anæsthetised animal runs less risk of septic infection and has, moreover, a shorter anæsthesia. In a small number of cases frogs were used, the injection being made directly into the cavity of the ventricle.

Results.

(*a*) *Intravascular admixture.*

Coarse quartz suspensions. Our earliest experiments, when no particular precautions were taken to protect against agglutination of the particles, seemed to indicate that coarse suspensions may cause coagulation, whereas fine suspensions do not. On repeating the work, however, with particles suspended in dextrose solution, we failed to obtain coagulation by injection of coarse quartz. Table I gives a record of 12 experiments, ten on rabbits and two on guinea-pigs, in which amounts

Table I. (Coarse quartz suspensions.)

	Dose in grm. per kg.	c.c. of fluid injected	Percentage of quartz in suspension	Remarks
Rabbit A	0·043	10	1·07	—
„ B	0·13	9	2·36	—
„ C	0·11	9	2·36	—
„ D	0·12	10	2·36	—
„ E	0·15	10	2·56	Death in 1 day
„ F	0·19	11	2·56	—
„ G	0·23	36	1·07	—
„ H	0·25	40	1·87	Killed for examination after 15 minutes
„ I	0·50	25	4·05	Death in 3½ hours
„ J	0·50	25	4·05	Death in 8 hours
Guinea-pig A	0·062	9	0·69	—
„ B	0·23	10	2·36	Death in 17 hours

of quartz varying from 0·04 to 0·5 grm. per kg. were injected. Even when the animals died as a result of the injection, *e.g.* rabbits I and J, the blood was found to be perfectly fluid. So, too, in rabbit H, which was killed 15 minutes after injection, no sign of thrombosis was discoverable.

Medium and fine suspensions. Experiments similar to those recorded in Table I were carried out on many occasions with medium and fine suspensions (cf. Elvidge(4)). In no case did intravascular coagulation result.

(*b*) *Extravascular admixture.*

Addition of quartz to drawn blood. In the preceding experiments the foreign particles were added to the circulating blood. We know, however, that direct addition, even of thrombin, to circulating blood fails to clot it, which means that in the body the blood is to some degree protected against influences which would otherwise induce coagulation. Consequently it was necessary to know whether admixture with quartz particles initiates or accelerates the coagulation of drawn blood. The blood used in such experiments must of course be shielded from any contact with other water-wettable foreign matter or with recently intermingled tissue-juice. Frog's blood, which is easy to handle, was first used. As it is difficult, if not impossible, to keep mammalian blood permanently fluid, we employed instead (1) cooled mammalian plasma, which has a full content of thrombocytes, and (2) negative phase mammalian plasma (prepared by repeated slow injections of dilute tissue-juice), which, owing to its low content in thrombocytes, clots very slowly on a paraffined surface (cf. Burke and Tait(3)).

From one and the same frog four samples of blood, consisting of two drops each, were removed and preserved in a moist chamber on successive paraffined watch-glasses after the method described by Tait and Green(16). Two of the samples (1 and 3) were kept as controls. To each of the other two samples (2 and 4) were added two drops of coarse quartz dextrose suspension. Each watch-glass in turn was thereupon agitated for a few seconds so as to roll the blood about. The result was as follows:

No. 1. No coagulation in 24 hours.
No. 2. Solid coagulation in 6 minutes.
No. 3. No coagulation in 24 hours.
No. 4. Solid coagulation in 5½ minutes.

These experiments were repeated on several occasions with similar findings. A pinch of dry (unlevigated) quartz particles dropped into

fluid frog's blood on paraffin also induces prompt coagulation. It is therefore plain that the drawn blood of the frog is induced to coagulate on contact with coarse quartz suspension.

By injecting directly into the ventricle we carried out the converse experiment of mingling coarse quartz with the circulating blood of the frog. In order to examine for thrombosis, which does not here produce sudden death as it does in the mammal, we sometimes watched the mesentery, at other times we displaced the blood by perfusion of the vessels. The results were not so uniformly negative as when quartz suspension is introduced into the circulatory current of mammals, and we did on some occasions produce thrombosis. At the same time both the dose administered and the rate of injection were relatively greater than in the corresponding mammalian experiments.

The effect of adding *fine* quartz suspension to drawn frog's blood is of special interest. Provided a very small quantity of the suspension is added, coagulation does not occur. If the quantity is increased, clotting results. The following experiments illustrate this fact:

(1) 3 drops blood + a minute drop of fine quartz suspension:
no coagulation in 2 hours and 40 minutes.
Large drop of fine quartz now added:
complete coagulation in 6½ minutes.

(2) 3 drops blood + a minute drop of fine suspension:
no coagulation in 1 hour and 47 minutes.
Large drop of fine quartz now added:
complete coagulation in 5½ minutes.

(3) 3 drops blood + a large drop of fine suspension:
complete coagulation in 11 minutes.

(4) 4 drops blood + a large drop of fine suspension:
partial coagulation in 3½ minutes;
complete coagulation in 30 minutes.

The simplest interpretation to put on these experiments is that the spindle-cells rupture only when they have engorged an excess of fine particles.

The experiments on the drawn blood of mammals were just as decisive in establishing coagulant action as those on the drawn blood of the frog. We shall first deal with the cooled plasma. On three occasions at different dates three samples each of cooled mammalian plasma, procured as described by Tait and Burke(15), were placed on paraffined watch-glasses at room temperature. One sample in each case acted as a control. To each of the other two, cooled medium quartz suspension was added. The results were as follows:

Exp. A:

1. Plasma control: clotted in 20 minutes.
2. Plasma + medium quartz: clotted in 7½ minutes.
3. Plasma + medium quartz: clotted in 5 minutes.

Exp. B:

1. Plasma control: no coagulation in 1 hour.
2. Plasma + medium quartz: clotted in 12 minutes.
3. Plasma + medium quartz: clotted in 10 minutes.

Exp. C:

1. Plasma control: clotted in 30 minutes.
2. Plasma + medium quartz: clotted in 7 minutes.
3. Plasma + dry quartz: clotted in 8 minutes.

In a second series of experiments centrifuged plasma obtained from negative phase blood and containing a reduced number of platelets was similarly treated, except that the temperature conditions were those of the room throughout. One example will suffice for illustration:

1. Plasma control: coagulation in 1 hour.
2. Plasma + medium quartz: clotted in 10 minutes.
3. Plasma + medium quartz: clotted in 12 minutes.

An interesting variation of the last experiment was to use plasma from blood which had been deprived of practically all its platelets. As Burke and Tait have shown, such blood can be obtained by giving rapidly a minimal lethal dose of highly active tissue extract. Two examples are given:

Exp. D:

1. Plasma control: no coagulation in 1 hour.
2. Plasma + medium quartz: no coagulation in 1 hour.

Exp. E:

1. Plasma control: no coagulation in 2 hours.
2. Plasma + medium quartz: filmy coagulation in 15 minutes; no further coagulation.

As proved by the firm coagulation that resulted from addition of tissue extract, both these samples of blood contained abundant fibrinogen.

The net conclusion from all these experiments is that admixture of quartz particles of sufficient size, or in sufficient amount if excessively fine, causes prompt coagulation of drawn blood, provided the thrombocytes are present. By contrast the negative effect of mingling the same particles with the circulating blood is rendered more striking.

(c) Fall in number of platelets on injection of particles.

Various workers have shown that when foreign particles are injected into the blood vessels of a mammal they are rapidly swept out of the blood stream. Thus in the experiments of Tait and McCartney(19), who injected Indian ink, the blood was found to be free of particles 1 hour after injection, and so were the organs with the constant exception of the spleen, liver and bone marrow, and with the occasional exception of the lung and of the kidney. It is naturally a hard matter to determine by simple inspection of the blood the presence or absence of quartz particles at any specified time after injection. There is, however, no doubt that they are rapidly removed, because they can be detected in sections of liver, spleen and bone marrow at least as early as 15 minutes after injection.

As our object was to look for some relation between the blood platelets and the injected particles, platelet counts were carried out at various intervals after injection. It was at once rendered plain that introduction of quartz into the vessels causes an abrupt fall in the number of circulating platelets. In this respect our findings were similar to those of Burke and Tait(3), who injected cell *débris*. Control experiments showed that the effect is in no way due to injection of glucose; it is wholly the result of mingling foreign particles with the blood. Soon after the initial fall the number of platelets begins to rise and after a period is again restored to normal.

For counting the platelets the method of Spitz(11) was used, the blood being diluted ten times. As a unit quantity of blood for enumeration we took the amount of the dilution that covered 16 squares of a Thoma-Zeiss hæmatocytometer. Some figures are appended representing the relative number of platelets in the blood before, and at different intervals after, injection of quartz, the experiments being grouped into three sets, according as enumerations were made at intervals of minutes, of hours or of days respectively after injection:

Set I. (Time in minutes.)

Weight of rabbit, 1865 grm.	Coarse quartz.	Dose, 0·5 grm. per kg. in 25 c.c. fluid.	
Initial count	250	After 3 mins.	55
Weight of rabbit, 1742 grm.	Fine quartz.	Dose, 0·3 grm. per kg. in 22 c.c. fluid.	
Initial count	274	After 10 mins.	28
After 3 mins.	21	„ 20 „	85

From the above two experiments it appears that whether a small quantity of fine or a larger quantity of coarse quartz is used, a prompt fall in the number of circulating platelets results.

Set II. (Time in hours.)

Weight of rabbit, 2460 grm.	Fine quartz.	Dose, 0·02 grm. per kg. in 20 c.c. fluid.	
Initial count	125	After 3 hours	75
After 3 mins.	27	„ 4 „	77
„ ½ hour	76		
Weight of rabbit, 1742 grm.	Fine quartz.	Dose, 0·03 grm. per kg. in 22 c.c. fluid.	
Initial count	273	After 24 hours ...	156
After 3 mins.	19	„ 48 „	152
„ 6 hours	120	„ 72 „	157
„ 12 „	137		
Weight of rabbit, 2500 grm.	Fine quartz.	Dose, 0·008 grm. per kg. in 7 c.c. fluid.	
Initial count	200	After ½ hour	152
After 3 mins.	156	„ 1⅓ hours	170
„ ¼ hour	160	„ 3½ „	200

The second experiment of the set shows that when a considerable quantity is injected recovery may still be incomplete after 3 days. In the third experiment, where, after a small dose, the initial fall was slight, recovery soon occurred.

Set III. (Time in days.)

Weight of rabbit, 1580 grm.	Medium quartz.	Dose, 0·32 grm. per kg. in 39 c.c. fluid.	
Initial count	200	After 4 days	98
After 1 day	96	„ 6 „	256
Weight of rabbit, 2040 grm.	Medium quartz.	Dose, 0·17 grm. per kg. in 27 c.c. fluid.	
Initial count	208	After 4 days	67
After 3 mins.	27	„ 7 „	200
„ 1 day	45	„ 9 „	256

In these instances recovery was in each case delayed for a number of days. As other experiments have shown, the ultimate over-stepping of the initial figure in these two experiments is not a constant finding.

As it was possible that the blood drawn from the marginal vein of the ear might not be representative of the systemic blood, we carried out an experiment in which the samples for examination were drawn simultaneously from the ear vein and from the carotid artery. The rabbit was anæsthetised with ether; its carotid artery was exposed and clamped with the fingers. The artery was incised and a small quantity of blood was removed for examination. Almost at the same time another sample was taken from the ear vein. The arterial sample showed 168 platelets per unit; the ear samples showed 144 per unit. Of medium suspension 0·14 grm. per kg. was now injected. Blood removed 3 minutes later from the carotid artery showed 46 platelets per unit; blood removed almost simultaneously from the ear vein showed 33. Fifteen and 30 minutes respectively after the first samples were taken, blood removed from the carotid artery showed 42 and 50 platelets per unit. By this experiment it was rendered plain that as far as number of platelets

is concerned blood taken from the ear is a sufficient index of the condition of the general systemic blood.

By repetition of the quartz injections the platelets can be kept at a low figure, but if the procedure is continued it leads to a fatal result. Thus in one experiment, in which the initial number was 138, a preliminary injection of 0·02 grm. per kg. of fine quartz caused an immediate drop to 30. Thereafter the platelet figure rose, being 79 at the end of $1\frac{1}{2}$ hours. At the end of 4 hours, when the figure was 86, another dose of similar amount was injected. This time there was no profound drop, the platelet count 3 minutes later registering 82. After a lapse of 21 hours from the time of the first injection, the platelet number was found to be 48. A dose of 0·005 grm. per kg. was now given, whereupon the figure fell to 22. A fourth injection, again of 0·005 grm. per kg., was administered 2 hours later. The animal soon showed marked weakness and died 3 hours later, its blood platelet number being very low.

The results cited above refer in each case to injections of quartz particles. Similar phenomena occur when other particles, *e.g.* barium, carmine or Indian ink, are substituted for quartz. As the particles can be shown to come to rest in the spleen, liver and bone marrow, and as a large number of platelets disappear simultaneously with the particles, the presumption is that the platelets, having ingested or having adhered to the particles, also come to rest in the same organs. It is a difficult matter to provide conclusive proof that the platelets which disappear have found a resting place in these localities. As some subsidiary evidence pointing in this direction we may mention that E. S. Mills, working in this laboratory on phagocytosis of Indian ink by the ellipsoids of the spleen, finds in these organs individual aggregations of ink of the size of platelets. The recovery in number of circulating platelets after an injection of foreign particles is, we believe, due to the appearance of a fresh crop.

(*d*) *Relation to coagulation.*

It remains to discuss the anomaly of the first two experimental sections, according to which extravascular admixture determines coagulation, whereas intravascular admixture, even with coarse particles, does not nearly so readily do so. The blood in circulation must in some way be protected against a coagulating influence which, if allowed access to drawn blood, is sufficient to induce irreversible solidification. The probability is that a partial transformation of fibrinogen into fibrin occurs in the circulating blood, but under conditions that preclude general solidification.

A closely similar anomaly arises in connection with the action of tissue extract or "thrombokinase." Direct addition of this material to drawn blood invariably causes coagulation, yet if the substance is very slowly injected into a vein the circulating blood stays fluid and soon exhibits impaired coagulability or "negative phase." Though it has failed to undergo solidification, such negative phase blood, as J. Mellanby(20) showed, has been wholly or partially defibrinated. Mellanby's explanation of the phenomenon is that when the kinase is slowly injected fibrin actually forms, but so gradually and in such small molecules that time and opportunity are allowed for its removal by means of the tissue cells. If the injection is more rapid, large cohering filaments form, which in turn implies a real thrombosis.

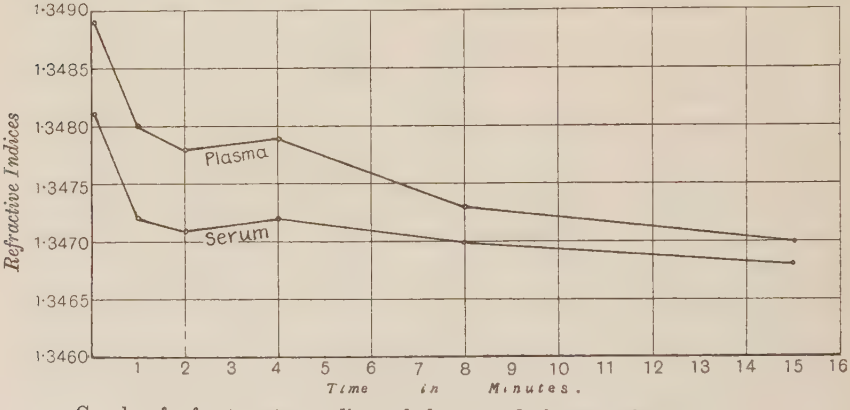
It is probable that the quartz injection, like a slow injection of thrombokinase, leads to transformation of fibrinogen into fibrin. Quartz, however, acts in a different way from thrombokinase. Whereas the latter directly coagulates fibrinogen (see Tait and Green(16)) the action of the quartz is indirect. It first causes cytolysis of platelets; owing to this cytolysis thrombin is liberated; the thrombin then acts upon the fibrinogen. It is open to suppose that the process of thrombin liberation may occur to a considerable extent in the blood stream. At the same time we have reason to believe that a large number of platelets, which have adhered and are about to cytolyse, are caught by the cells and other structures of the reticulo-endothelial system, whereby they may be put out of action, so far at least as power to cause generalised coagulation is concerned. In any case, owing to its indirect mode of action, an injection of quartz particles must operate on fibrinogen more slowly than a corresponding injection of thrombokinase.

At some particular point, say, 10–20 minutes, after a single injection of quartz, and while abundant particles are still floating free in the blood, the coagulation time of the blood is found to be greatly shortened. This can be determined by pulling a needle through the drawn blood and noting the moment of appearance of the first thread of fibrin, which, at this stage, may be almost immediate! This striking fact supports the idea that thrombin is then being liberated freely within the circulating blood. So rapid is the formation of fibrin at this stage that we had difficulty in understanding how the blood keeps fluid in the vessels. We judged that the animal had reached a danger-point, at which the vascular mechanism for counteracting thrombosis was being called into strenuous activity. This in turn throws additional light on the difference in action between an injection of tissue extract and one of

quartz. In the case of tissue extract the outstanding experimental difficulty is to inject so slowly as to avoid, at any stage, sudden overloading of the mechanism for counteracting thrombosis. With quartz it is the other way about; one may approach, but one does not readily reach the overloading point.

At a later stage, say, after 40 minutes, the coagulation time becomes much greater than normal, and the drawn blood is slow to clot on glass and other water-wettable receptacles. It does, however, coagulate solidly in the end, which proves that it still contains fibrinogen. The delay in spontaneous coagulation at this stage is no doubt associated with the reduction in number of platelets, and perhaps with a difference in physiological state of the new crop as compared with the old.

In order to discover whether fibrinogen is removed from the blood as a result of quartz injection, we had recourse to the refractometer. At different stages after injection samples of blood were withdrawn. These were divided into two portions. One was first oxalated and then centrifuged in order to provide plasma. The other, which was left to clot, provided the serum. The accompanying figure represents in graphical



Graphs of refractometer readings of plasma and of serum after an injection of quartz particles.

form the result of one of these experiments. The abscissa represents time in minutes after injection; the ordinates represent the refractometer readings. The upper trace shows readings of the plasma, the lower trace readings of the serum. It will be seen that during the first minute there is in each case a sharp and equal fall of the respective refractive indices, due to blood dilution. Thereafter the course of each graph is more nearly linear. During this second stage the refractive index of the serum falls

off slightly, probably owing to successive withdrawals of blood samples. That of the plasma, on the other hand, falls more rapidly; at the end of 15 minutes it registers only 0·0002 above the serum, whereas at the moment of injection it stood 0·0008 above the serum. This plainly indicates a loss of fibrinogen from the plasma.

Just as Mellanby had to account for the disappearance of fibrinogen in negative phase blood on the assumption that fibrin had actually been formed and removed from the blood, so we have here to postulate the activity of a definite anti-thrombotic *mechanism*, as opposed to a material "anti-thrombin." As Mellanby pointed out, conditions tending towards thrombosis, *e.g.* death or injury of blood cells, are continually arising in the blood. Should the organism be unable to deal with a slow (or, we may add, dispersed) formation of fibrin in its circulating blood, thrombosis would be a continual occurrence in the life-history of animals. We shall not here attempt to define the mechanism in question or to specify its conditions of operation. Suffice it to say that the reason why a direct injection of thrombin into the circulation fails to cause thrombosis, whereas injection of thrombokinase is effective, probably depends upon the difficulty of securing an adequate concentration of thrombin to overwhelm for the moment the anti-thrombotic mechanism.

It may be mentioned that during the course of this investigation one of the authors found that injection of particles affects not only the number of platelets, but also the number and kind of red cells in circulation (see Elvidge(4)).

Summary.

1. *A priori* considerations seemed to indicate that an injection of very fine inert particles might fail to cause coagulation of the circulating blood, whereas an injection of coarse particles might cause coagulation. It was found that an intravascular injection even of coarse particles does not necessarily determine thrombosis.

2. Addition of coarse particles to paraffin-held, drawn blood, by causing cytolysis of thrombocytes, eventually causes coagulation. Addition of an adequate quantity of fine particles to the drawn blood of the frog similarly causes coagulation, while addition of a smaller quantity of fine particles does not.

3. As the result of an intravascular injection of particles a fall in the number of circulating platelets occurs. After a heavy injection the fall is profound (though complete disappearance was never observed), and some days must elapse before the previous number is restored. With

injection of a smaller amount the fall in number of platelets is less, and the time for recovery of the initial number is shorter. By repetitions of the injections at intervals less than the time necessary for recovery, the number of circulating platelets can be kept low.

4. Admixture of particles with the circulating blood, while failing to cause thrombosis, nevertheless effects a transformation of fibrinogen into fibrin. The reason why thrombosis does not occur is that the particular conditions of formation of the fibrin allow of a successful reply on the part of the natural "anti-thrombotic mechanism" of the circulation.

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REFERENCES.

1. Bedson. Lancet, 207. pp. 1117–9. 1924.
2. Bredig. Zeitschr. f. Elektrochem. Jahrg. 4. pp. 514–5, 544–6. 1897–8. Quoted from Wolfgang Ostwald's Handbook of Colloid Chemistry. Transl. by M. H. Fischer, Blakiston, Philadelphia, 1915, p. 135. 1898.
3. Burke and Tait. Quart. Journ. Exp. Physiol. 16. pp. 111–27. 1926.
4. Elvidge. Amer. Journ. Path. 2. pp. 189–91. 1926.
5. Govaerts. La Presse médicale, No. 65. 25 Nov. 1918; Arch. internat. de physiol. 16. pp. 1–20. 1921.
6. Hardy. This Journ. 13. pp. 165–90. 1892.
7. Heim. Études sur le sang des Crustacés décapodes. Thèse, Paris, Imprimerie Édouard Duruy, pp. 1–92. 1892.
8. Kraus and Dexter. Journ. Amer. Chem. Soc. 44. pp. 2468–71. 1922.
9. Meves. Arch. f. mikr. Anat. 68. pp. 311–58. 1906.
10. Rettger. Amer. Journ. Physiol. 24. pp. 406–35. 1909.
11. Spitz. Berlin klin. Wochenschr. Jahrg. 58. pp. 1073–5. 1921.
12. Tait. Quart. Journ. Exp. Physiol. 3. pp. 1–20. 1910.
13. Tait. Journ. Mar. Biol. Assoc. 9. pp. 191–8. 1911.
14. Tait. Quart. Journ. Exp. Physiol. 12. pp. 1–33. 1918.
15. Tait and Burke. Ibid. 16. pp. 129–40. 1926.
16. Tait and Green. Ibid. 16. pp. 141–8. 1926.
17. Tait and Gunn. Ibid. 12. pp. 35–80. 1918.
18. Zsigmondy. Liebig's Ann. d. Chem. 301. pp. 29–54, esp. p. 33. 1898.
19. Tait and McCartney. This Journ. 53. Proc. Physiol. Soc. p. xxii. 1919.
20. Mellanby. This Journ. 38. pp. 441–503 (especially p. 475). 1909.